L2: Entry 9 of 10 File: USPT Jan 31, 1989

DOCUMENT-IDENTIFIER: US 4801688 A
TITLE: Hydrazone immunoglobulin conjugates

Abstract Text (1):
Immunoglobulin conjugates formed by reaction of an antineoplastic indole-dihydroindole vinca alkaloid containing a hydrazine group attached at C-3 or C-4 with an oxidized glycoprotein containing aldehyde groups.

Brief Summary Text (2):
Immunoglobulins are glycoproteins, i.e., oligosaccharides are attached to the protein at various sites. Vicinal diols of these oligosaccharides can be oxidized with periodate to yield dialdehydes, and the aldehyde groups thus produced reacted with various amines and hydrazines to form Schiff bases and hydrazones. For example, Willan et al., FEBS Letters, 80 133 (1977) oxidized an oligosaccharide attached to an asparagine residue at position 297 (using the numbering system of the human IgG1 myeloma protein Eu) in the C.sub.H 2 region of rabbit IgG. This oxidized material was, after purification, reacted with 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl free radical and the resulting imine (reaction of amino group with sugar aldehyde) reduced with NaBH.sub.3 CN to yield a spin labelled protein. Timofeev et al., FEBS Letters, 89 191 (1978) also used oxidized glycoprotein monoclonal antibodies to prepare spinlabeled material. Murayama et al., Immunochemistry, 15 523 (1978) labeled periodate oxidized oligosaccharidegroups in an immunoglobulin (IgG) with amino-containing compounds via Schiff base formation. Aspartic acid and horseradish peroxidase were the "amines" employed. The aspartic acid-Schiff base was used to detect the antigenicity of human IgG by counter immunoelectrophoresis. et al., Immunology Letters, 8 273 (1984), coupled biotin hydrazide with aldehyde groups of oxidized immunoglobulins and suggested the use of the procedure for conjugation of fluorescent dyes to monoclonal antibodies.

Brief Summary Text (23):
This invention provides conjugates formed by reaction of an oxidized glycoprotein containing one or more aldehyde groups with a vinca hydrazide. The hydrazide group can be either a C-3 carboxhydrazide (COR in formula I below) or a C-4 hydrazide-containing ester linked (R.sup.6 in formula I below) via a hydrocarbon chain.

Brief Summary Text (24):
The vinca portion of the conjugate is described by Formula I below. The conjugates of this invention are formed by reaction of a compound of structure I with the aldehyde groups of an oxidized glycoprotein. ##STR1## wherein R.sup.2 is H, CH.sub.3 or CHO; when R.sup.4 and R.sup.5 are taken singly, R.sup.5 is H, and one of R.sup.3 and R.sup.4 is ethyl and the other is H or OH; when R.sup.4 and R.sup.5 are taken together with the carbons to which they are attached, they form an oxirane ring in which case R.sup.3 is ethyl; R is NHNH.sub.2, O(C.sub.1 -.sub.3 alkyl), NH.sub.2, NH(C.sub.1 -.sub.3 alkyl), NH-CH.sub.2 CH.sub.2 -Y, 1-pyrrolidinyl or 1-piperidinyl, wherein n is 2-4 and Y is Cl, OCH.sub.3 or SCH.sub.3 ; R.sub.1 is H, (C .sub.1 -.sub.3 alkyl)-CO, chloro-substituted (C.sub.1 -.sub.3 alkyl)-CO or R.sup.6 wherein R.sup.6 is COXCONHNH.sub.2 wherein X is C.sub.1 -.sub.4 straight chain alkylene, C.sub.2 -.sub.8 branched chain alkylene, C.sub.2 -.sub.4 alkenylene, C.sub.3 -.sub.4 alkynylene, C.sub.3 -.sub.6 cycloalkylene, phenylene, hydroxysubstituted C.sub.1 -.sub.4 alkylene, or a direct bond, except that R cannot be NHNH.sub.2 when R.sup.1 is R.sup.6 and R.sup.1 cannot be R.sup.6 when R is NHNH.sub.2. Groups illustrative

of X in the above formulas include methylene, ethylene, propylene, butylene, vinyl, propylene, butenylene, butynylene, propynylene, hydroxyethylene, 1,2-dihydroxyethylene, 1,2-dimethylethylene, 1,2,3,4-tetrahydroxybutylene, 3,4-dimethylbutylene, 1,4-cyclohexylene, 1,4-phenylene, 1,2-phenylene, and the like.